AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning at page 2, line 11, as follows.

--FIG. 1 is a graph wherein (A) shows Agarose gel electrophoresis of low density lipoprotein (hereinafter referred to as "LDL"). LDL was incubated with $CuSO_4$ or Sodium Nitroprusside (hereinafter referred to as "SNP") for 24 hours at 37**°C**. The sample on the gel lanes are follows: lane 1, native LDL; lane 2, LDL incubated with $CuSO_4$; lane 3-5, LDL incubated with $CuSO_4$ in the presence of 0.5 (lane 3), 1 (lane4) and 2 mg/ml(lane5) Hibiscus sabdariffa extract (hereinafter referred to as "HSE"); lane 6, LDL incubated with SNP; lane7-9, LDL incubated with SNP in the presence of 0.5 (lane7), 1 (lane8) and 2 mg/ml(lane9) HSE; (B) shows Determination of relative ~~electrophretic~~ **electrophoretic** mobility. * LDL degradation;--

Please amend the paragraph beginning at page 2, line 20, as follows.

--FIG. 2 is a graph wherein (A) shows Agarose gel electrophoresis of LDL. LDL was incubated with $CuSO_4$ for 24 hours at 37**°C**. The sample on the gel lanes are follows: lane 1, native LDL; lane 2, LDL incubated with $CuSO_4$; lane 3-5, LDL incubated with $CuSO_4$ in the presence of 0.5 (lane3), 1 (lane4), 2 mg/ml(lane5) HSE; lane 6, LDL

pre-incubated with $CuSO_4$; lane 7-9, LDL pre-incubated with $CuSO_4$ than addition of 0.5 (lane7), 1 (lane8) and 2 mg/ml(lane9) HSE; (B) shows Determination of relative ~~electrophretic~~ **electrophoretic** mobility;

Please replace the paragraph beginning at page 2, line 26, with the following amended paragraph.

--FIG. 3 is a table showing ~~Pre treative~~ **Pretreatment** effect of HSE on the $CuSO_4$ induced lipid peroxidation in LDL. * $p<0.001$; ** $p<0.00001$, compared with CuSO.sub.4 treated LDL group;--

Please replace the paragraph beginning at page 3, line 1, with the following amended paragraph.

-- FIG. 4 is a table showing ~~Post treative~~ **Post-treatment** effect of HSE on the $CuSO_4$ induced lipid peroxidation in LDL. * $p<0.01$; ** $p<0.0001$, compared with CuSO.sub.4 treated alone group;--

Please replace the paragraph beginning at page 3, line 27 with the following amended paragraph.

--FIG. 11 is a table showing Effect of HSE on hepatic function in rabbit and rat[a] Abbreviation, ALT, alanine transaminase; AST, ~~asparate~~ **aspartate** aminotransferase; ALP, alkaline phosphatase;--

Please replace the paragraph beginning at page 4, line 22, with the following amended paragraph.

--The molecular weight of LDL is smaller when LDL has not been oxidated, therefore, the mobility is greater in the SDS-PAGE experiment under the same potential. Comparatively, the molecular weight of LDL is larger when LDL has been ~~oxidated~~ **oxidized**, therefore, the mobility is lower in the SDS-PAGE experiment under the same potential. After LDL being incubated with 10 **μM** ~~M~~ $CuSO_4$ or 100 mM SNP for 24 hours at 37**°C** respectively in the presence of various concentrations of HSE, it is observed that the higher the concentration of HSE is, the more significantly the oxidation of LDL is countered. The results are shown in FIG. 1.--

Please replace the paragraph beginning at page 4, line 22, with the following amended paragraph.

--The molecular weight of LDL is smaller when LDL has not been oxidated, therefore, the mobility is greater in the SDS-PAGE experiment under the same potential. Comparatively, the molecular weight of LDL is larger when LDL has been oxidated, therefore, the mobility is lower in the SDS-PAGE experiment under the same potential. After LDL being incubated with 10 M $CuSO_4$ or 100 mM SNP for 24 hours at 37**°C** respectively in the presence of various concentrations of HSE, it is observed that the higher the

concentration of HSE is, the more significantly the oxidation of LDL is countered. The results are shown in FIG. 1.—

Please replace the paragraph beginning at page 5, line 1, with the following amended paragraph.

--We can learn from the above that the oxidation of LDL incubated with $CuSO_4$ or SNP will be countered in the presence of HSE. It is also observed that adding HSE after LDL was pre-incubated with $CuSO_4$ for 24 hours at 37°C is helpful in countering the oxidation of LDL. In addition, the higher the concentration of HSE is, the more significantly the oxidation of LDL is countered. The results are shown in FIG. 2.—-

Please replace the paragraph beginning at page 5, line 6, with the following amended paragraph.

--Additionally, LDL were pretreated with various concentrations of HSE for 5 minutes, and then incubated with 10 μM $CuSO_4$ for 24 hours at 37°C. Comparing with the control group, the measurements of the TBARs indicate that pretreatment with HSE can significantly reduce the formation of TBARs, the group pretreated with 1 mg/dl HSE outperformed the control group by 180% ($P<0.01$), the group pretreated with 2 mg/dl HSE outperformed the control group by 1800% ($P<0.00001$). The results are shown in FIG. 3.--

Please replace the paragraph beginning at page 5, line 12, with the following amended paragraph.

--Further, LDL were incubated with $CuSO_4$ 10 μM HSE for 5 minutes, and then post-treated with various concentrations of HSE for 24 hours at 37**°C**. The measurements of the TBARs indicate that post-treatment with HSE is able to decrease the formation of TBARs, the group post-treated with 1 mg/dl HSE outperformed the control group by 190% ($P<0.01$), the group post-treated with 2 mg/dl HSE outperformed the control group by 1700% ($P<0.0001$). The results are shown in FIG. 4.--

Please replace the paragraph beginning at page 5, line 18, with the following amended paragraph.

--Cu^{2+} may induce the oxidation of LDL and then cause Apo B fragmentation in LDL. LDL (120 g/ml) was incubated with 10 **μM** ~~M~~ $CuSO_4$ at 37**°C** in the presence of HSE for 4 hours. After the incubation, EDTA (final concentration 1 mM) was added to prevent any further oxidation. Approximately 6 mg protein of the LDL was applied to SDS-PAGE (315% gradient). After the electrophoresis, each spot was stained with Coomassie Brilliant blue R250. M, standard molecular weight markers. It is observed that the higher the concentration of HSE is, the less the Apo B fragmentation in

LDL is caused, i.e., the higher the concentration of HSE is, the more significantly the oxidation of LDL is countered. The results are shown in FIG. 5.--

REMARKS

Claims 1, 5-7 and 14 are pending in this application.

The specification has been reviewed and amended as indicated above, to correct readily apparent typographical errors. No new matter has been added with the amendments.

Rejections under 35 U.S.C. § 112, 1st paragraph

Claim 14 has been rejected under 35 U.S.C. § 112, 1st paragraph as lacking written description of "inhibiting" artherosclerosis. As noted previously, page 2, second paragraph of the specification clearly describes that the inventors contemplated as part of their invention, the inhibition of artherosclerosis. In the present application, the unexpected, advantageous effect of *Hibiscus sabdariffa* extract (HSE) for inhibiting oxidation of LDL is shown in Example 1 and FIGS. 1-5. In addition, experimental data of the specification demonstrates a lower lipid deposit in the endothelium cells of the arteriae and no foam cells being formed in rabbits that were fed with a cholesterol-fed diet with HSE. See Example 3 and FIGS. 9-10. These experiments demonstrate that atherosclerosis is "inhibited" by administering an effective amount of HSE. As such, the invention as claimed is fully supported by the specification.

Rejections under 35 U.S.C. § 102

Claims 1, 5-7 and 12 have been rejected under 35 U.S.C. § 102 as being anticipated by Brink.

Claims 1, 5, 6, and 14 have been rejected under 35 U.S.C. § 102 as being anticipated by CN 1156552, JP 56029522 or FR 2454277.

Claims 1, 5, and 14 have been rejected under 35 U.S.C. § 102 as being anticipated by JP 2000095663, JP 2000239164, JP 09295928, JP 2000154134 or Ibnusaud et al.

Claims 1, 5, and 14 have been rejected under 35 U.S.C. § 102 as being anticipated by Clarke et al.

Applicants traverse these rejections and withdrawal thereof is respectfully requested. "To anticipate a claim, a prior art reference must disclose every limitation of the claimed invention..." In re Schreiber, 128 F.3d 1473, 1477, 44 USPQ2d 1429, 1431 (Fed. Cir. 1997).

The present invention, as encompassed by amended claim 1 is drawn to a method of inhibiting oxidation of low density lipoproteins which comprises administering an effective amount of a Hibiscus sabdariffa extract to a patient in need thereof; wherein oxidation of low density lipoproteins is inhibited by the Hibisucus sabdariffa extract. Thus, the present invention requires the inhibition of oxidation of low density lipoproteins. As noted previously and as will be shown below in more detail, none of the

references disclose a method for the inhibition of oxidation of low density lipoproteins.

1) Brink - Brink discloses a weight control composition made from a mixture of guggul extract, particularly from *Commiphora mukul* or *Commiphora wightii*, and at least one phosphate salt selected from calcium phosphate, potassium phosphate and sodium phosphate. The guggul extract/phosphate salt is used to reduce plasma lipid levels and cholesterol in overweight hyperlipidaemic humans. The present invention discloses a method for inhibiting the oxidation of LDL and inhibiting atherosclerosis by administering an effective amount of a *Hibiscus sabdariffa* extract. There is no suggestion in Brink of an extract from *Hibiscus sabdariffa*. As such, the present invention is clearly not anticipated by Brink.

2) CN 1156552, JP 56029522 or FR 2454277 - Claims 1, 5, 6, 14 are rejected under 35 U.S.C. 102(b) for being anticipated by CN 1156552, JP 56029522 or FR 2454277. All three references teach an orally administered composition. CN 1156552 is directed to a process for manufacturing jelly and beverages from *Hibiscus sabdariffa Linn.* With FR 2454277, leaves extract of KarKade or

Karak are used to prepare beverages and in JP 56029522, a tea bag of *Hibiscus sabdariffa* and the manufacturing method thereof is disclosed. Thus, these references Beverages and tea bags are kinds of drinks and these references do not teach inhibiting oxidation of LDL as disclosed in the present invention.

3) *JP 2000095663, JP 2000239164, JP 09295928, JP 2000154134 or Ibnusaud et al.* - In JP2000095663 discloses a plant extract or a combination of plant extracts for external use such as a skin whitening agent and an anti-microbial agent applied in the field of cosmetics and quasi-drugs. Thus, JP '663 discloses a topical formulation. JP09295928 similarly discloses a topical cosmetic material for the improvement of a tenseness/wrinkle of a skin, containing *Hibiscus Sabdariffa L.* itself or an extract thereof. A topical cosmetic containing 3-hydroxy-3,4-dicarboxy-1,4-butanolide obtained by extracting calyxes of *Hibiscus sabdariffa* is also disclosed in JP2000154134. The cosmetic can improve aging of the skin but it does not relate to the effect of inhibiting oxidation of LDL.

In JP2000239164, a glycosidase inhibitor extracted from a plant belonging to the genus Hibiscus is disclosed. The glycosidase inhibitor is used for drug to treat diabetes mellitus but it is

irrelevant to both inhibiting the oxidation of LDL and inhibiting atherosclerosis.

Ibnusaud et al. discloses a process for isolating Hibiscus acid from the leaves of *Hibiscus sabdariffa*. There is no disclosure in Ibnusaud et al. regarding pharmacological activity.

3) Clarke et al. - Clarke et al. disclose cultured plant cell gums, particular of the genus *Mesembryanthemum*, to manufacture food, pharmaceutical, cosmetic and industrial products such as an emulsifying agent, coating agent, thickening agent, stabilizer, etc. The disclosure of Clarke et al. is irrelevant to inhibiting of oxidation of LDL and atherosclerosis using *Hibiscus sabdariffa*.

In summary, none of the cited references teaches or discloses inhibiting oxidation of LDL of *Hibiscus sabdariffa* extract and inhibiting atherosclerosis by administering an effective amount of *Hibiscus sabdariffa* extract. As such, none of the references disclose the presently claimed invention and withdrawal of the rejections is respectfully requested.

Rejection under 35 U.S.C. § 103

Claims 1, 5-7 and 14 have been rejected under 35 U.S.C. § 103 as being obvious over JP 2000095663, JP 2000239164, JP 09295928, JP 2000154134, Ibnusaud et al., Clarke et al., CN 1156552, JP 56029522

or FR 2454277 combined with Brink. The primary references are asserted to teach a composition and Brink is asserted to teach the composition in the form of a tablet. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

As stated above, none of the cited references teach or suggest inhibiting oxidation of LDL with an *Hibiscus sabdariffa* extract and inhibiting atherosclerosis by administering an effective amount of *Hibiscus sabdariffa* extract. The present invention discloses for the first time a newly discovered use of *Hibiscus sabdariffa,* which is novel and non-obvious to the public.

The combination of the references is considered improper because the present invention is not achieved by the combination. Brink teaches a method for reducing the plasma lipid levels and/or cholesterol levels in a mammal with a composition of guggul extract and at least one phosphate salt. Brink specifically teaches in the *"Background of the invention"* that the reduction of cholesterol level is from the combination the guggulsterone with a mixture of phosphate salts. With the present invention, inhibiting oxidation of LDL is due to the highly anti-oxidant activity of the components of *Hibiscus sabdariffa,* an entirely different mechanism.

None of the references disclose a method for the inhibition of oxidation of low density lipoproteins. As such, all of the

references are fatally flawed and the invention cannot be achieved by combining the references.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong (Reg. No. 40,069) at the telephone number of the undersigned below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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